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(54) Title: METHOD FOR MAKING HUMANIZED ANTIBODIES

(57) Abstract

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

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METHOD FOR MAKING HUMANIZED ANTIBODIES

Field of the Invention

This invention relates to methods for the preparation and use of variant antibodies and finds application particularly in the fields of immunology and cancer diagnosis and therapy.

Background of the Invention

Naturally occurring antibodies (immunoglobulins) comprise two heavy chains linked together by disulfide bonds and two light chains, one light chain being linked to each of the heavy chains by disulfide bonds. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain (V_L) at one end and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia *et al., J. Mol. Biol.* 186:651-663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. USA* 82:4592-4596 (1985).

The constant domains are not involved directly in binding the antibody to an antigen, but are involved in various effector functions, such as participation of the antibody in antibody-dependent cellular cytotoxicity. The variable domains of each pair of light and heavy chains are involved directly in binding the antibody to the antigen. The domains of natural light and heavy chains have the same general structure, and each domain comprises four framework (FR) regions, whose sequences are somewhat conserved, connected by three hyper-variable or complementarity determining regions (CDRs) (see Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, MD, (1987)). The four framework regions largely adopt a β-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site.

Widespread use has been made of monoclonal antibodies, particularly those derived from rodents including mice, however they are frequently antigenic in human clinical use. For example, a major limitation in the clinical use of rodent monoclonal antibodies is an anti-globulin response during therapy (Miller, R. A. *et al., Blood* 62:988-995 (1983); Schroff, R. W. *et al., Cancer Res.* 45:879-885 (1985)).

The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a human constant domain (Cabilly *et al.*, U.S. patent No. 4,816,567; Morrison, S. L. *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Bouliann , G. L. *et al., Nature* 312:643-646 (1984); Neuberger, M.

S. *et al.*, *Nature* 314:268-270 (1985)). The term "chimeric" antibody is used herein to describe a polypeptide comprising at least th antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

The isotype of the human constant domain may be selected to tailor the chimeric antibody for participation in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (see e.g. Brüggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987); Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Love *et al.*, *Methods in Enzymology* 178:515-527 (1989); Bindon *et al.*, *J. Exp. Med.* 168:127-142 (1988).

In the typical embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody, OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers, G. J. *et al.*, *Transplantation* 41:572-578 (1986)).

In a further effort to resolve the antigen binding functions of antibodies and to minimize the use of heterologous sequences in human antibodies, Winter and colleagues (Jones, P. T. *et al.*, *Nature* 321:522-525 (1986); Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Verhoeyen, M. *et al.*, *Science* 239:1534-1536 (1988)) have substituted rodent CDRs or CDR sequences for the corresponding segments of a human antibody. As used herein, the term "humanized" antibody is an embodiment of chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The therapeutic promise of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Hale, G. *et al.*, *Lancet* i:1394-1399 (1988)). A murine antibody to the interleukin 2 receptor has also recently been humanized (Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)) as a potential immunosuppressive reagent. Additional references related to humanization of antibodies include Co *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2869-2873 (1991); Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4181-4185 (1991); Daugherty *et al.*, *Nucleic Acids Research* 19(9):2471-2476 (1991); Brown *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2663-2667 (1991); Junghans *et al.*, *Cancer Research* 50:1495-1502 (1990).

In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones, P. T. *et al.*, *Nature* 321:522-525 (1986); Verhoeyen, M. *et al.*, *Science* 239:1534-1536 (1988)), whereas in

other cases it has been n cessary to additionally replace one (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988)) or several (Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)) framework region (FR) residues. See also Co *et al.*, *supra*.

For a given antibody a small number of FR residues are anticipated to be important for antigen binding. Firstly for example, certain antibodies have been shown to contain a few FR residues which directly contact antigen in crystal structures of antibody-antigen complexes (e.g., reviewed in Davies, D. R. *et al.*, *Ann. Rev. Biochem.* 59:439-473 (1990)). Secondly, a number of FR residues have been proposed by Chothia, Lesk and colleagues (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987); Chothia, C. *et al.*, *Nature* 342:877-883 (1989); Tramontano, A. *et al.*, *J. Mol. Biol.* 215:175-182 (1990)) as critically affecting the conformation of particular CDRs and thus their contribution to antigen binding. See also Margolies *et al.*, *Proc. Natl. Acad. Sci. USA* 72:2180-2184 (1975).

It is also known that, in a few instances, an antibody variable domain (either V_H or V_L) may contain glycosylation sites, and that this glycosylation may improve or abolish antigen binding, Pluckthun, *Biotechnology* 9:545-51 (1991); Spiegelberg *et al.*, *Biochemistry* 9:4217-4223 (1970); Wallic *et al.*, *J. Exp. Med.* 168:1099-1109 (1988); Sox *et al.*, *Proc. Natl. Acad. Sci. USA* 66:975-982 (1970); Margni *et al.*, *Ann. Rev. Immunol.* 6:535-554 (1988). Ordinarily, however, glycosylation has no influence on the antigen-binding properties of an antibody, Pluckthun, *supra*, (1991).

The three-dimensional structure of immunoglobulin chains has been studied, and crystal structures for intact immunoglobulins, for a variety of immunoglobulin fragments, and for antibody-antigen complexes have been published (see e.g., Saul *et al.*, *Journal of Biological Chemistry* 25:585-97 (1978); Sheriff *et al.*, *Proc. Natl. Acad. Sci. USA* 84:8075-79 (1987); Segal *et al.*, *Proc. Natl. Acad. Sci. USA* 71:4298-4302 (1974); Epp *et al.*, *Biochemistry* 14(22):4943-4952 (1975); Marquart *et al.*, *J. Mol. Biol.* 141:369-391 (1980); Furey *et al.*, *J. Mol. Biol.* 167:661-692 (1983); Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267-279, Alan R. Liss, Inc. pubs. (1986); Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987); Chothia *et al.*, *Nature* 342:877-883 (1989); Chothia *et al.*, *Science* 233:755-58 (1986); Huber *et al.*, *Nature* 264:415-420 (1976); Bruccoleri *et al.*, *Nature* 335:564-568 (1988) and *Nature* 336:266 (1988); Sherman *et al.*, *Journal of Biological Chemistry* 263:4064-4074 (1988); Amzel and Poljak, *Ann. Rev. Biochem.* 48:961-67 (1979); Silverton *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5140-5144 (1977); and Gregory *et al.*, *Molecular Immunology* 24:821-829 (1987). It is known that the function of an antibody is dependent on its three dimensional structure, and that amino acid substitutions can change the three-dimensional structure of an antibody, Snow and Amzel, *supra*. It has previously b en shown that the antigen binding affinity of a humanized antibody can be increased by mutagen sis based upon molecular modelling (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)).

Humanizing an antibody with retention of high affinity for antigen and other desired biological activities is at present difficult to achieve using currently available procedures. Methods are needed for rationalizing the selection of sites for substitution in preparing such antibodies and thereby increasing the efficiency of antibody humanization.

The proto-oncogene *HER2* (human epidermal growth factor receptor 2) encodes a protein tyrosine kinase ($p185^{HER2}$) that is related to and somewhat homologous to the human epidermal growth factor receptor (see Coussens, L. *et al.*, *Science* **230**:1132-1139 (1985); Yamamoto, T. *et al.*, *Nature* **319**:230-234 (1986); King, C. R. *et al.*, *Science* **229**:974-976 (1985)). *HER2* is also known in the field as *c-erbB-2*, and sometimes by the name of the rat homolog, *neu*. Amplification and/or overexpression of *HER2* is associated with multiple human malignancies and appears to be integrally involved in progression of 25-30% of human breast and ovarian cancers (Slamon, D. J. *et al.*, *Science* **235**:177-182 (1987), Slamon, D. J. *et al.*, *Science* **244**:707-712 (1989)). Furthermore, the extent of amplification is inversely correlated with the observed median patient survival time (Slamon, *supra*, Science 1989).

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al.*, *Cancer Res.* **50**:1550-1558 (1990)), directed against the extracellular domain (ECD) of $p185^{HER2}$, specifically inhibits the growth of tumor cell lines overexpressing $p185^{HER2}$ in monolayer culture or in soft agar (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* **9**:1165-1172 (1989); Lupu, R. *et al.*, *Science* **249**:1552-1555 (1990)). MuMAb4D5 also has the potential of enhancing tumor cell sensitivity to tumor necrosis factor, an important effector molecule in macrophage-mediated tumor cell cytotoxicity (Hudziak, *supra*, 1989; Shepard, H. M. and Lewis, G. D. *J. Clinical Immunology* **8**:333-395 (1988)). Thus muMAb4D5 has potential for clinical intervention in and imaging of carcinomas in which $p185^{HER2}$ is overexpressed. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published 27 July 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. However, this antibody may be immunogenic in humans.

It is therefore an object of this invention to provide methods for the preparation of antibodies which are less antigenic in humans than non-human antibodies but have desired antigen binding and other characteristics and activities.

It is a further object of this invention to provide methods for the efficient humanization of antibodies, i.e. selecting non-human amino acid residues for importation into a human antibody background sequence in such a fashion as to retain or improve the affinity of the non-human donor antibody for a given antigen.

It is another object of this invention to provide humanized antibodies capable of binding $p185^{HER2}$.

Other objects, features, and characteristics of the present invention will become apparent upon consideration of the following description and the appended claims.

Summary of the Invention

The objects of this invention are accomplished by a method for making a humanized antibody comprising amino acid sequence of an import, non-human antibody and a human antibody, comprising the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L - V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, the method of this invention comprises the additional steps of determining if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), retaining the consensus residue.

Additionally, in certain embodiments the method of this invention comprises the feature wherein the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)).

In certain embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid

residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another embodiment of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

Certain alternate embodiments of the methods of this invention comprise obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

In preferred embodiments, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody.

Optionally, this just-recited embodiment comprises the additional steps of following the method steps appearing at the beginning of this summary and determining whether a particular amino acid residue can reasonably be expected to have undesirable effects.

This invention also relates to a humanized antibody comprising the CDR sequence of an import, non-human antibody and the FR sequence of a human antibody, wherein an amino acid residue within the human FR sequence located at any one of the sites 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H has been substituted by another residue. In preferred embodiments, the residue substituted at the human FR site is the residue found at

the corr sponding location of the non-human antibody from which the non-human CDR was obtained. In other embodiments, no human FR residue other than those set forth in this group has been substituted.

This invention also encompasses specific humanized antibody variable domains, and isolated polypeptides having homology with the following sequences.

1. SEQ. ID NO. 1, which is the light chain variable domain of a humanized version of muMAb4D5:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLESGVP SRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQTKVEIKRT

2. SEQ. ID NO. 2, which is the heavy chain variable domain of a humanized version of muMAb4D5):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLV TVSS

In another aspect, this invention provides a consensus antibody variable domain amino acid sequence for use in the preparation of humanized antibodies, methods for obtaining, using, and storing a computer representation of such a consensus sequence, and computers comprising the sequence data of such a sequence. In one embodiment, the following consensus antibody variable domain amino acid sequences are provided:

SEQ. ID NO. 3 (light chain):

DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLESGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENGGYT RYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTL VTVSS

Brief Description of the Drawings

FIGURE 1A shows the comparison of the V_L domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus sequence (Fig. 1A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIGURE 1B shows the comparison between the V_H domain amino acid residues of the muMAb4d5, huMAb4D5, and a consensus sequence (Fig. 1B, SEQ. ID NO. 6, SEQ. ID NO. 2 and SEQ. ID NO. 4, respectively). Both Figs 1A and 1B use the generally accepted numbering scheme from Kabat, E. A., *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD (1987)). In both Fig. 1A and Fig. 1B, the CDR residues determined according to a standard sequence definition (as

in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) are indicated by the first underlining beneath the sequences, and the CDR residues determined according to a structural definition (as in Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)) are indicated by the second, lower underlines. The mismatches between genes are shown by the vertical lines. FIGURE 2 shows a scheme for humanization of muMAb4D5 V_L and V_H by gene conversion mutagenesis.

FIGURE 3 shows the inhibition of SK-BR-3 proliferation by MAb4D5 variants. Relative cell proliferation was determined as described (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* 9:1165-1172 (1989)) and data (average of triplicate determinations) are presented as a percentage of results with untreated cultures for muMAb4D5 (l), huMAb4D5-8 (n) and huMAb4D5-1 (l).

FIGURE 4 shows a stereo view of α-carbon tracing for a model of huMAb4D5-8 V_L and V_H. The CDR residues (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) are shown in bold and side chains of V_H residues A71, T73, A78, S93, Y102 and V_L residues Y55 plus R66 (see Table 3) are shown.

FIGURE 5 shows an amino acid sequence comparison of V_L (top panel) and V_H (lower panel) domains of the murine anti-CD3 monoclonal Ab UCHT1 (muxCD3, Shalaby *et al.*, *J. Exp. Med.* 175, 217-225 (1992) with a humanized variant of this antibody (huxCD3v9). Also shown are consensus sequences (most commonly occurring residue or pair of residues) of the most abundant human subgroups, namely V_L κ 1 and V_H III upon which the humanized sequences are based (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest*, 5th edition, National Institutes of Health, Bethesda, MD, USA (1991)). The light chain sequences--muxCD3, huxCD3v9 and huκI--correspond to SEQ.ID.NOs 16, 17, and 18, respectively. The heavy chain sequences--muxCD3, huxCD3v9 and huκI--correspond to SEQ.ID.NOs 19, 20, and 21, respectively. Residues which differ between muxCD3 and huxCD3v9 are identified by an asterisk (*), whereas those which differ between humanized and consensus sequences are identified by a sharp sign (#). A bullet (o) denotes that a residue at this position has been found to contact antigen in one or more crystallographic structures of antibody/antigen complexes (Kabat *et al.*, 1991; Mian, I. S. *et al.*, *J. Mol. Biol.* 217, 133-151 (1991)). The location of CDR residues according to a sequence definition (Kabat *et al.*, 1991) and a structural definition (Chothia and Lesk, *supra* 1987) are shown by a line and carats (^) beneath the sequences, respectively.

FIGURE 6A compares murine and humanized amino acid sequences for the heavy chain of an anti-CD18 antibody. H52H4-160 (SEQ. ID. NO. 22) is the murine sequence, and pH52-8.0 (SEQ. ID. NO. 23) is the humanized heavy chain sequence. pH52-8.0 residue 143S is the final amino acid in the variable heavy chain domain V_H, and residue 144A is the first amino acid in the constant heavy chain domain C_{H1}.

FIGURE 6B compares murine and humanized amino acid sequences for the light chain of an anti-CD18 antibody. H52L6-158 (SEQ. ID. NO. 24) is the murine sequence, and pH52-9.0 (SEQ. ID. NO. 25) is the humanized light chain sequence. pH52-9.0 residue 128T is the final amino acid in the light chain variable domain V_L, and residue 129V is the first amino acid in the light chain constant domain C_L.

FIGURE 7A shows an amino acid sequence alignment of the sequences of the heavy chains of thirteen humanized anti-CD18 (H52) variants (SEQ. ID. NOs 26-38).

FIGURE 7B shows an amino acid sequence alignment of two humanized anti-CD18 (H52) light chain variants (SEQ. ID. NOs 39-40).

Detailed Description of the Invention

Definitions

In general, the following words or phrases have the indicated definitions when used in the description, examples, and claims:

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al.*, *Cancer Res.* 50:1550-1558 (1990)) is directed against the extracellular domain (ECD) of $p185^{HER2}$. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published 27 July 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. In this description and claims, the terms muMAb4D5, chMAb4D5 and huMAb4D5 represent murine, chimerized and humanized versions of the monoclonal antibody 4D5, respectively.

A humanized antibody for the purposes herein is an immunoglobulin amino acid sequence variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a FR region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin.

Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are referred to herein as "import" residues, which are typically taken from an "import" antibody domain, particularly a variable domain. An import residue, sequence, or antibody has a desired affinity and/or specificity, or other desirable antibody biological activity as discussed herein.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will

contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain.

The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, e.g., the import CDR or the consensus FR may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or FR residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 90%, and most preferably greater than 95%.

In general, humanized antibodies prepared by the method of this invention are produced by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen.

Residues that influence antigen binding are defined to be residues that are substantially responsible for the antigen affinity or antigen specificity of a candidate immunoglobulin, in a positive or a negative sense. The invention is directed to the selection and combination of FR residues from the consensus and import sequence so that the desired immunoglobulin characteristic is achieved. Such desired characteristics include increases in affinity and greater specificity for the target antigen, although it is conceivable that in some circumstances the opposite effects might be desired. In general, the CDR residues are directly and most substantially involved in influencing antigen binding (although not all CDR residues are so involved and therefore need not be substituted into the consensus sequence). However, FR residues also have a significant effect and can exert their influence in at least three ways: They may noncovalently directly bind to antigen, they may interact with CDR residues and they may affect the interface between the heavy and light chains.

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr
230 232

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Gly Ile Asn Pro Lys Asn Gly Gly Thr Ser His
50 55 60

Asn Gln Arg Phe Met Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 100 105

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser L u Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr
230 232

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Gly Ile Asn Pro Lys Asn Gly Gly Thr Ser His
50 55 60

Asn Gln Arg Phe Met Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 100 105

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr
230 232

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Gly Ile Asn Pro Lys Asn Gly Gly Thr Ser His
50 55 60

Asn Gln Arg Phe Met Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 100 105

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr
230 232

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Lys Gln Ser His Gly Lys Ser Leu
35 40 45

Glu Trp Val Ala Gly Ile Asn Pro Lys Asn Gly Gly Thr Ser His
50 55 60

Asn Gln Arg Phe Met Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 100 105

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr
230 232

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Ile Gly Gly Phe Asn Pro Lys Asn Gly Gly Thr Ser His
50 55 60

Asn Gln Arg Phe Met Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 100 105

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr
230 232

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 214 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Asn
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Tyr Thr Ser Thr Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Gly Asn Thr Leu Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys
214

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 214 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Asn
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Gly Asn Thr Leu Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys
214

CLAIMS

WE CLAIM:

1. A method for making a humanized antibody comprising amino acid sequence of a non-human, import antibody and a human antibody, comprising the steps of:
   a. obtaining the amino acid sequences of at least a portion of an import variable domain and of a consensus human variable domain;
   b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;
   c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
   d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
   e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
   f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:
      1. non-covalently binds antigen directly,
      2. interacts with a CDR; or
      3. participates in the V_L - V_H interface; and
   g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.
2. The method of claim 1, having an additional step of determining if any such non-homologous residues are exposed on the surface of the domain or buried within it, and if the residue is exposed, retaining the consensus residue.
3. The method of claim 1, having the additional steps of searching the import variable domain sequence for glycosylation sites, determining if any such glycosylation site is reasonably expected to affect the antigen binding or affinity of the antibody, and if so, substituting the glycosylation site into the consensus sequence.
4. The method of claim 1, having the additional steps of searching the consensus variable domain sequence for glycosylation sites which are not present at the corresponding amino acid in the import sequence, and if the glycosylation site is not present in the import sequence, substituting the import amino acid residues for the amino acid residues comprising the consensus glycosylation site.
5. The method of claim 1, having an additional step which comprises aligning import antibody and consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if th

corresponding consensus antibody residue represents a residue which is highly conserv d across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

6. The method of claim 1, wherein the corresponding consensus antibody residues are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

7. A method comprising providing at least a portion of an import, non-human antibody variable domain amino acid sequence having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:
4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

8. The method of claim 7, wherein the substituted residue is the residue found at the corresponding location of the non-human antibody.

9. The method of claim 1 or 7, wherein the consensus human variable domain is a consensus based on human variable domains and additionally variable domains from species other than human.

10. A humanized antibody variable domain having a non-human CDR incorporated into a human antibody variable domain, wherein the improvement comprises substituting an amino acid residue for the human residue at a site selected from the group consisting of:
4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

11. The humanized antibody variable domain of claim 10, wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained.

12. The humanized antibody variable domain of claim 10, wherein no human FR residue other than those set forth in the group has been substituted.

13. A polypeptide comprising the amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLESGVP
SRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT

14. A polypeptide comprising the sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLV
TVSS

15. A method for engineering a humanized antibody comprising introducing amino acid residues from an import antibody variable domain into an amino acid sequence representing a consensus of mammalian antibody variable domain sequences.

16. A computer comprising the sequence data of the following amino acid sequence:
a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLE
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or
b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISE
NGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
MDVWGQGTLVTVSS

17. A computer representation of the following amino acid sequence:
a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLE
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or
b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISE
NGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
MDVWGQGTLVTVSS

18. A method comprising storing a computer representation of the following amino acid sequence:
a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLE
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or
b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISE
NGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
MDVWGQGTLVTVSS

FIG. 1A

```
                10        20        30        40        50
4D5     DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAVAWYQQKPGHSPKLLIYSASFRYT
HU4D5   DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLES
HUVLκI  DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLES
                ----------  ------          --      --------
                VL-CDR1                     VL-CDR2

                60        70        80        90        100
4D5     GVPDRFTGNRSGTDFTFTISSVQAEDLAVYYCQQHYTTPPTFGGGTKLEIKRA
HU4D5   GVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT
HUVLκI  GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT
                          ---------
                          VL-CDR3
```

FIG. 1B

```
                10        20        30        40        50  A
4D5     EVQLQQSGPELVKPGASLKLSCTASGFNIKDTYIHWVKQRPEQGLEWIGRIYPTN
                ||  ||  |  |  |  |  |  |  |  |  |  |  |  |  ||
HU4D5   EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTN
                   |||  ||||  |  |  |  |  |  |  |  |  |  |||||
HUVHIII EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENG
                   ----------  ----------  ----------  -------
                               VH-CDR1     VH-CDR2

                60        70        80  ABC   90        100ABC
4D5     GYTRYDPKFQDKATITADTSSNTAYLQVSRLTSEDTAVYYCSRWGGDGFYAMDYW
                |||||||||  |  |  |  |  |||  ||  |  |  |  |  |  |
HU4D5   GYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVW
        ||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
HUVHIII SDTYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGGAVSYFDVW
        ----------  ----------  ----------  ----------  -------
                                            VH-CDR3

                110
4D5     GQGASVTVSS
                ||
HU4D5   GQGTLVTVSS

HUVHIII GQGTLVTVSS
```

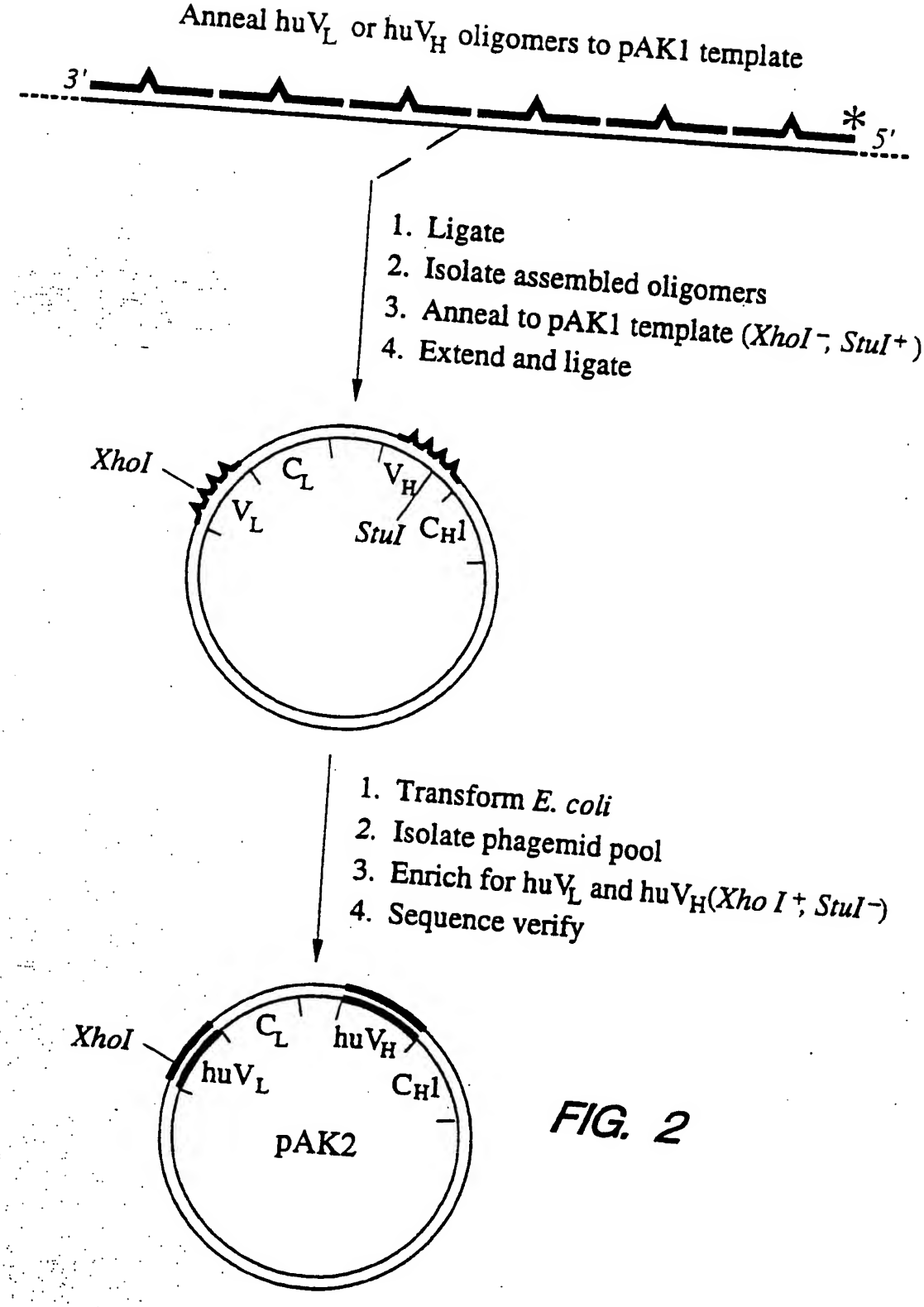
Anneal huV$_L$ or huV$_H$ oligomers to pAK1 template
3'
5'
1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI*$^-$, *StuI*$^+$)
4. Extend and ligate
XhoI
C$_L$
V$_H$
V$_L$
StuI
C$_H$1
1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV$_L$ and huV$_H$(*Xho I*$^+$, *StuI*$^-$)
4. Sequence verify
XhoI
C$_L$
huV$_H$
huV$_L$
C$_H$1
pAK2

FIG. 2

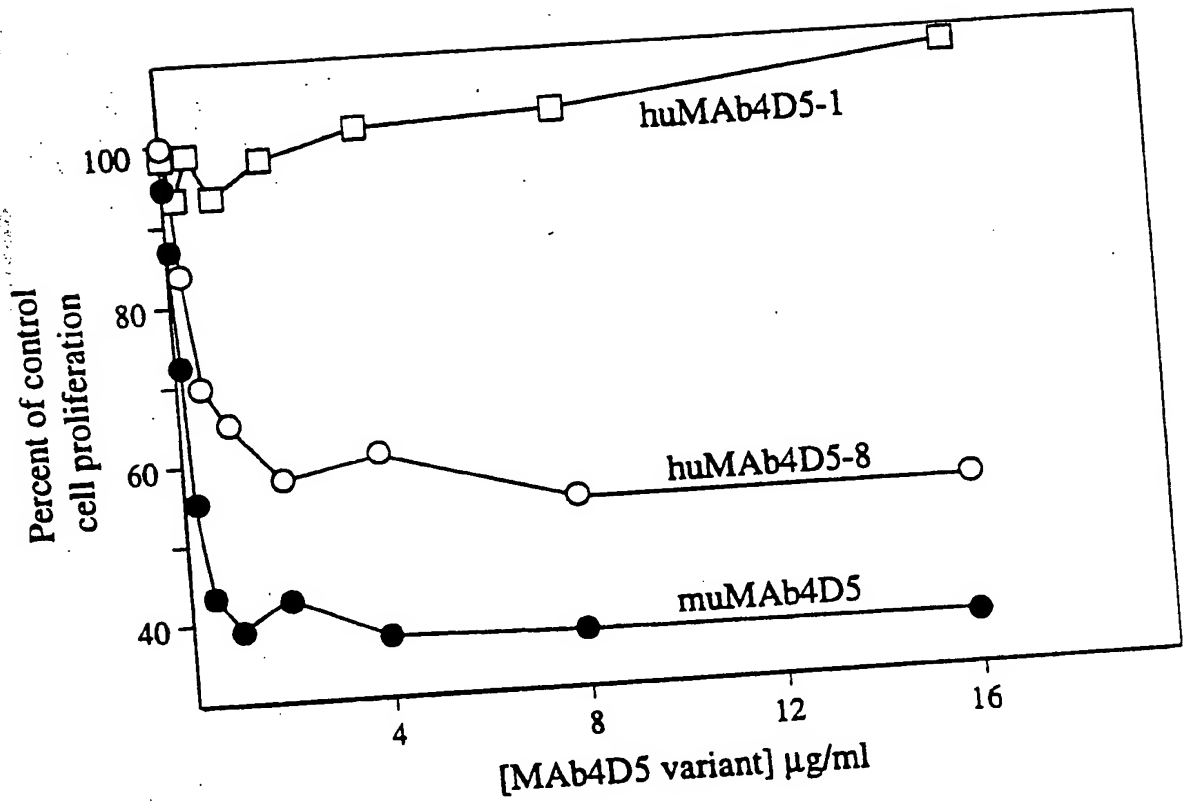
huMAb4D5-1
huMAb4D5-8
muMAb4D5
100
80
60
40
Percent of control cell proliferation
4
8
12
16
[MAb4D5 variant] µg/ml

FIG. 3

FIG. 4

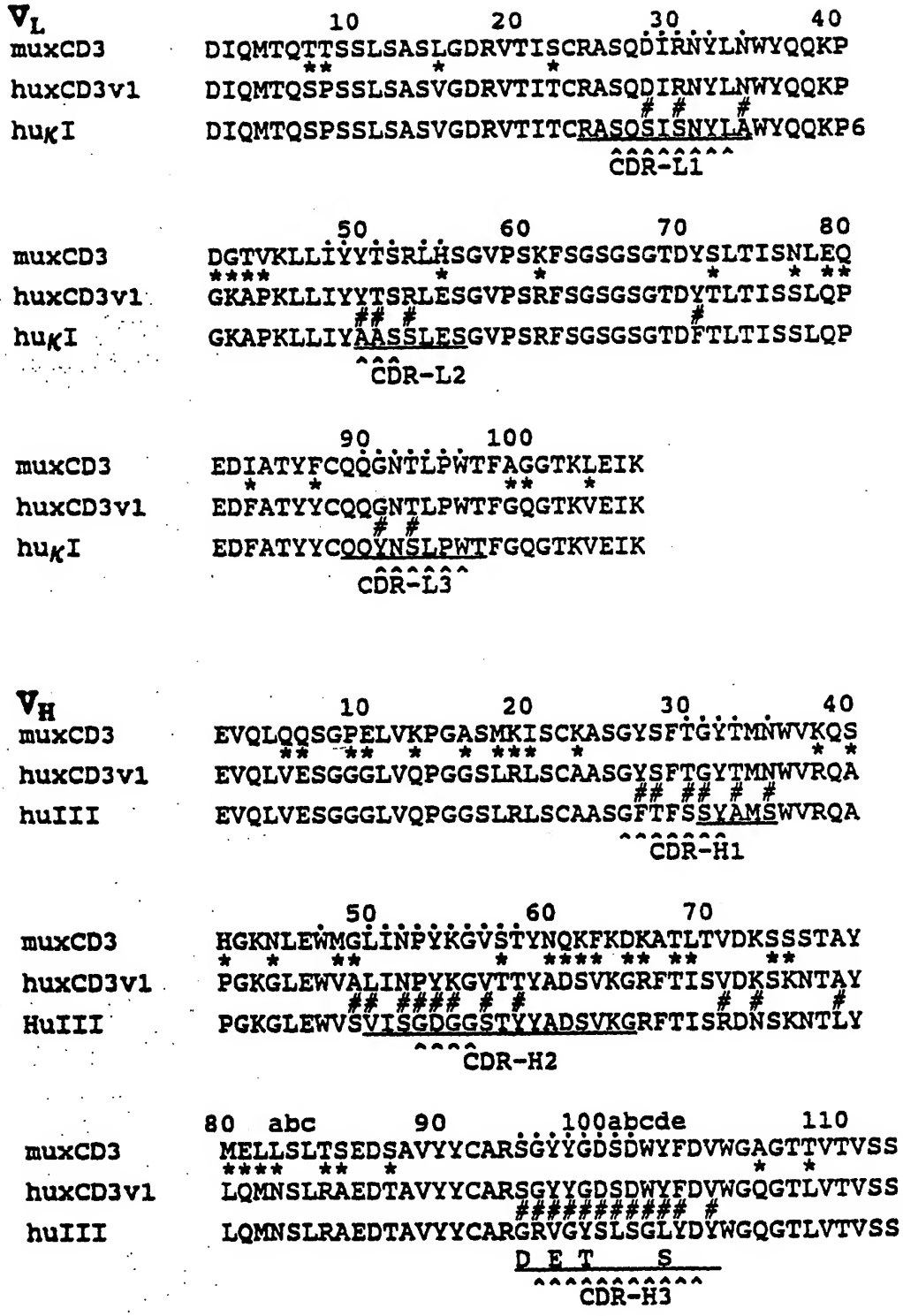

FIG. 5

SUBSTITUTE SHEET

FIG. 6A-1

```
                                10                  20                  30
H52H4-160                       QVQLQQSGPELVKPGASVKISCKTSGYTFTE
                                .*** .**  **.**.*...** ********
pH52-8.0        MGWSCIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCATSGYTFTE
                          10        20        30        40        50

                        40        50        60        70        80
H52H4-160       YTMHWMKQSHGKSLEWIGGFNPKNGGSSHNQRFMDKATLAVDKSTSTAYM
                ******.*. **.***..*.*******.********. *..**********
pH52-8.0        YTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISVDKSTSTAYM
                          60        70        80        90       100

                        90       100       110       120       130
H52H4-160       ELRSLTSEDSGIYYCARWRGLNYGFDVRYFDVWGAGTTVTVSSASTKGPS
                .. ** .**...**********************  ** **********
pH52-8.0        QMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPS
                         110       120       130       140       150

                       140       150       160       170       180
H52H4-160       VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
                ****** *.*** .**********************************
pH52-8.0        VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
                         160       170       180       190       200

                       190       200       210       220       230
H52H4-160       QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
                ********** **..*****  ***.********** ** * *
pH52-8.0        QSSGLYSLSSVVTVTSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC---V
                         210       220       230       240

                       240       250       260       270       280
H52H4-160       TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
                *******  ..**********************************.
pH52-8.0        ECPPCPAPP-VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
                250       260       270       280       290
```

FIG. 6A-2

```
                290       300       310       320       330
H52H4-160 FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
          ********.**********.***.**********.**********
pH52-8.0  FNWYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
            300       310       320       330       340

                340       350       360       370       380
H52H4-160 NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
          **.**********.**********.**********.**********
pH52-8.0  NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
            350       360       370       380       390

                390       400       410       420       430
H52H4-160 SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
          **********.**********.**********.**********
pH52-8.0  SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
            400       410       420       430       440

                440       450
H52H4-160 CSVMHEALHNHYTQKSLSLSPGK
          **********.**********
pH52-8.0  CSVMHEALHNHYTQKSLSLSPGK
            450       460
```

FIG. 6B

```
                                    10        20        30
H52L6-158                           DVQMTQTTSSLSASLGDRVTINCRASQDINN
                                    *.****. ******.****** **********
pH52-9.0      MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASQDINN
                     10        20        30        40        50

                     40        50        60        70        80
H52L6-158     YLNWYQQKPNGTVKLLIYYTSTLHSGVPSRFSGSGSGTDYSLTISNLDQE
              **********  . **********************.****.*. *
pH52-9.0      YLNWYQQKPGKAPKLLIYYTSTLHSGVPSRFSGSGSGTDYTLTISSLQPE
                     60        70        80        90        100

                     90        100       110       120       130
H52L6-158     DIATYFCQQGNTLPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
              *.***.************ **********************************
pH52-9.0      DFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
                     110       120       130       140       150

                     140       150       160       170       180
H52L6-158     VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
              **********************************************************
pH52-9.0      VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
                     160       170       180       190       200

                     190       200       210
H52L6-158     SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
              **********************************
pH52-9.0      SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
                     210       220       230
```

FIG. 7A-1

```
verA.hcfab[1]    1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verI.hcfab[2]    1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verN.hcfab[3]    1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verO.hcfab[4]    1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verO.hcfab2[5]   1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verP.hcfab[6]    1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verQ.hcfab[7]    1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verR.hcfab[8]    1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verS.hcfab[9]    1 EVQLQQSGPELVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verT.hcfab[10]   1 EVQLVESGGGLVKPGASLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAG
verU.hcfab[11]   1 EVQLVESGGGLVQPGGSLKISCKTSGYTFTEYTMHWMRQAPGKGLEWVAG
verV.hcfab[12]   1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMKQSHGKSLEWVAG
verW.hcfab[13]   1 EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWIGG

verA.hcfab      51 INPKNGGTSYADSVKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR
verI.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR
verN.hcfab      51 INPKNGGTSHNQRFMDRFTLAVDKSKNTLYLQMNSLRAEDTAVYYCARWR
verO.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSTSTAYMQMNSLRAEDTAVYYCARWR
verO.hcfab2     51 INPKNGGTSHNQRFMDRFTISVDKSTSTAYMQMNSLRAEDTAVYYCARWR
verP.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSKNTLYMELRSLRAEDTAVYYCARWR
verQ.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSKNTLYLQMNSLTSEDSGIYYCARWR
verR.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR
verS.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR
verT.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR
verU.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR
verV.hcfab      51 INPKNGGTSHNQRFMDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR
verW.hcfab      51 FNPKNGGTSHNQRFMDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR

verA.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verI.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verN.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verO.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verO.hcfab2    101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verP.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verQ.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verR.hcfab     101 GLNYGFDVRYFDVWGAGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verS.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verT.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verU.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verV.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
verW.hcfab     101 GLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
```

1 SEQ.ID.NO.26
2 SEQ.ID.NO.27
3 SEQ.ID.NO.28
4 SEQ.ID.NO.29
5 SEQ.ID.NO.30
6 SEQ.ID.NO.31
7 SEQ.ID.NO.32
8 SEQ.ID.NO.33
9 SEQ.ID.NO.34
10 SEQ.ID.NO.35
11 SEQ.ID.NO.36
12 SEQ.ID.NO.37
13 SEQ.ID.NO.38

FIG. 7A-2

```
verA.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verI.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verN.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verO.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verO.hcfab2 151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verP.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verQ.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verR.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verS.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verT.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verU.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verV.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
verW.hcfab  151 CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL

verA.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verI.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verN.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verO.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verO.hcfab2   201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
verP.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verQ.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verR.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verS.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verT.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verU.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verV.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
verW.hcfab    201 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT---
```

FIG. 7B

```
                10        20        30        40        50
verAlc[1]  DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYY
verZlc[2]  DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYY

                60        70        80        90        100
verAlc     TSTLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQ
verZlc     TSTLHSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQ

               110       120       130       140       150
verAlc     GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
verZlc     GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV

               160       170       180       190       200
verAlc     DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
verZlc     DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG

               210
verAlc     LSSPVTKSFNRGEC
verZlc     LSSPVTKSFNRGEC
```

1. (SEQ.ID.NO.39)

2. (SEQ.ID.NO.40)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/07832

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/13 C12P21/08 C07K13/00 C12N5/10 G06F15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K C12N G06F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF MOLECULAR BIOLOGY vol. 215 , 1990 , ACADEMIC PRESS pages 175 - 182 Tramontano, Anna; Chothia, Cyrus; Lesk, Arthur M. 'Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins' cited in the application See the whole document, especially paragraph 7	1-12,15
Y	WO,A,90 07861 (PROTEIN DESIGN LABS, INC.) 26 July 1990 See pages 1-6; 9-25	1-12,15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 1993	01 -02- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nauche, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATURE. vol. 342 , December 1989 , LONDON GB pages 877 - 883 Chothia, Cyrus; Lesk, Arthur M.; Tramontano, Anna; Levitt, Michael; Smith-Gill, Sandra J.; Air, Gillian; Sheriff, Steven; Padlan, 'Conformations of immunoglobulin hypervariable region' cited in the application See the whole document, especially 'Discussion'	1-12,15
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89 , May 1992 , WASHINGTON US pages 4285 - 4289 Carter, Paul et al. 'Humanization of an anti-p185HER2 antibody for human cancer therapy.' see the whole document	1-18
P,X, L	WO,A,92 22653 (GENENTECH, INC.; US) 23 December 1992 see the whole document	1-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07832

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 17 - 18 (Rule 39.I.vi. PCT) - Program for computers.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 93/07832

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9007861	26-07-90	AU-A-	5153290	13-08-90
		CA-A-	2006865	28-06-90
		EP-A-	0451216	16-10-91
WO-A-9222653	23-12-92	AU-A-	2250992	12-01-93